

**AMENDMENTS TO THE CLAIMS**

**Listing of Claims**

1. (original) A method for producing a composition for the prevention or control of coccidiosis comprising:

collecting manure from host animals wherein said manure contains oocysts known to cause coccidiosis;

diluting said manure in an aqueous medium to create a slurry;

separating unwanted fecal matter from said slurry and collecting the aqueous fraction containing oocysts;

subjecting said aqueous fraction to solid/liquid phase centrifugal-based separation and collecting the solid phase;

combining a dense aqueous liquid with said collected solid phase wherein said dense liquid has a density greater than about 1.09 g/ml and wherein the oocysts are buoyant;

subjecting the combination of said dense aqueous liquid and collected solid phase to centrifugation and collecting the dense liquid fraction containing oocysts;

diluting said dense liquid fraction to a specific gravity wherein the oocysts are no longer buoyant;

separating oocyst solids from said diluted liquid fraction by centrifugal-based separation and re-collecting the solid phase.

2. (currently amended) A method as set forth in claim [[1]] 6 further comprising:

diluting said re-collected solid phase in an aqueous sporulation medium;

sporulating said oocysts while in contact with said sporulation medium;

separating sporulated oocysts from said sporulation medium;

sterilizing said sporulated oocysts; and

diluting said sporulated oocysts to form a vaccine composition.

3. (original) A method of separating oocysts from a liquid suspension by the use of a hydrocyclone.

4. (original) A method as set forth in claim 3 wherein the oocysts are collected in the underflow from the hydrocyclone.

5. (original) A method for isolating oocysts comprising:  
collecting manure from host animals wherein said manure contains oocysts known to cause coccidiosis;

diluting said manure in an aqueous medium to create a slurry;

separating unwanted fecal matter from said slurry and collecting the aqueous fraction containing oocysts;

subjecting said aqueous fraction to solid/liquid phase centrifugal-based separation by means of a hydrocyclone.

6. (currently amended) A method for isolating oocysts comprising:

collecting manure from host animals wherein said manure contains oocysts known to cause coccidiosis;

diluting said manure in an aqueous medium to create a slurry;

separating unwanted fecal matter from said slurry and collecting the aqueous fraction containing oocysts;

subjecting said aqueous fraction to solid/liquid phase centrifugal-based separation and collecting the solid phase;

combining a dense aqueous liquid with said collected solid phase wherein said dense liquid has a density greater than about 1.09 g/ml and wherein the oocysts are buoyant;

subjecting the combination of said dense aqueous liquid and collected solid phase to centrifugation and collecting the dense liquid fraction containing oocysts;

diluting said dense liquid fraction to a specific gravity wherein the oocysts are no longer buoyant;

separating oocyst solids from said liquid ~~phase~~ fraction by means of a hydrocyclone and re-collecting the solid phase.

7. (original) A method for isolating oocysts comprising:  
collecting feces from animals wherein said feces contains oocysts known to cause coccidiosis;  
contacting said feces with an aqueous medium;  
separating unwanted fecal matter from said oocysts;  
subjecting said oocysts to centrifugal-based separation and collecting the oocyst-containing solid fraction;  
suspending the oocyst-containing solid fraction in a flotation solution;

allowing the oocysts to separate from the solids, wherein the oocysts are floated to the top of the solution; and removing the flotation medium from said oocysts by tangential flow filtration.

8. (currently amended) A method as set forth in claim **[[1]]** 6 wherein said animals comprise the class Aves.

9. (original) A method as set forth in claim 8 wherein said slurry is created by mixing manure and water in relative proportions of from about 0.5 gallons to about 5 gallons of domestic water per the amount of manure obtained in about 3 days from about six animals comprising the class Aves.

10. (original) A method as set forth in claim 9 wherein said animals are chickens.

11. (currently amended) A method as set forth in claim **[[1]]** 6 wherein said separation of unwanted fecal matter comprises sieving.

12. (original) A method as set forth in claim 11 wherein said sieving is by the use of multiple-tier shaker screens.

13. (original) A method as set forth in claim 12 wherein said shaker screens comprise a 50-mesh screen and a 250-mesh screen.

14. (original) A method as set forth in claim 1 wherein said method is carried out at a temperature between about 4° C and about 30° C.

15. (original) A method as set forth in claim 14 wherein said sieving is carried out at a temperature between about 22° C and about 28° C.

16. (original) A method as set forth in claim 15 wherein said sieving is carried out at about 25° C.

17. (currently amended) A method as set forth in claim ~~[[1]]~~ 6 wherein ~~each of~~ said centrifugal-based separations comprises the use of a centrifuge or a ~~hydrocyclone~~ hydrocyclone.

18. (original) A method as set forth in claim 17 wherein said centrifugal-based separation comprises the use of a hydrocyclone.

19. (original) A method as set forth in claim 18 wherein said centrifugal-based separation comprises the use of a centrifuge.

20. (original) A method as set forth in claim 19 wherein said centrifuge is a bottle centrifuge.

21. (original) A method as set forth in claim 19 wherein said centrifuge is a continuous centrifuge.

22. (currently amended) A method as set forth in claim ~~[[1]]~~ 6 wherein said centrifugation is a bottle centrifuge.

23. (currently amended) A method as set forth in claim ~~[[1]]~~ 6 wherein said dense aqueous liquid comprises a solution of corn syrup or sodium chloride.

24. (currently amended) A method as set forth in claim ~~[[1]]~~ 6 wherein said dense aqueous ~~solution~~ liquid has a density from about ~~1.07~~ 1.09 g/ml to about 1.20 g/ml.

25. (currently amended) A method as set forth in claim 24 wherein said dense aqueous ~~solution~~ liquid has a density from about ~~1.08~~ 1.09 g/ml to about 1.14 g/ml.

26. (currently amended) A method as set forth in claim 25 wherein said dense aqueous ~~solution~~ liquid has a density from about 1.09 g/ml to about 1.10 g/ml.

27. (original) A method for inducing sporulation of oocysts comprising:

introducing into an aqueous sporulation medium oocysts of at least one species of protozoa known to cause coccidiosis;

incubating said oocysts in said aqueous sporulation medium, thereby causing sporulation of oocysts; and

introducing an oxidizing agent into said medium at a rate sufficient to maintain the average dissolved oxygen content during sporulation at at least 30% of saturation.

28. (original) A method as set forth in claim 27 wherein said dissolved oxygen content is substantially maintained at between about 30% and about 80% of saturation throughout the period of sporulation.

29. (original) A method as set forth in claim 28 wherein said dissolved oxygen content of the medium is substantially maintained at between about 40% and about 60% of saturation throughout the period of sporulation.

30. (original) A method as set forth in claim 29 wherein said dissolved oxygen content of the medium is substantially maintained at about 50% of saturation.

31. (original) A method as set forth in claim 27 wherein the alkali metal dichromate content of said sporulation medium is less than about 0.8% by weight during incubation of oocysts.

32. (original) A method as set forth in claim 27 comprising addition to said sporulation medium of an oxidizing agent having a standard reduction potential of at least about 0.5 V.

33. (original) A method as set forth in claim 32 comprising addition of both molecular oxygen and another oxidizing agent.

34. (original) A method as set forth in claim 33 wherein said oxidizing agent has a standard reduction potential of at least about 0.5 V.

35. (original) A method as set forth in claim 34 wherein said oxidizing agent is selected from the group consisting of an alkali metal hypochlorite, an alkali metal chlorite, an alkali metal chlorate, an alkali metal perchlorate, and an alkali metal permanganate.

36. (original) A method as set forth in claim 35 wherein said oxidizing agent comprises hypochlorite ions.

37. (original) A method as set forth in claim 35 wherein a sufficient amount of an alkali metal hypochlorite is added to achieve an alkali metal hypochlorite weight percent from about 0.001 weight percent to about 0.1 weight percent of the sporulation medium and oocysts combined, wherein said alkali metal hypochlorite is from about 1.0 % to about 10.0 % by volume.

38. (original) A method as set forth in claim 27 further comprising:

separating sporulated oocysts from said sporulation medium;



sterilizing sporulated oocysts by contacting said sporulated oocysts with a chemical disinfectant; and storing said sporulated oocysts in a sterile diluent, said diluent containing less than about 0.8% by weight alkali metal dichromate.

39. (original) A method as set forth in claim 27 wherein said medium contains less than about 0.3% by weight dichromate ion during incubation of said oocysts.

40. (original) A method as set forth in claim 27 wherein said medium contains less than about 0.15% by weight hexavalent chromium during incubation of said oocysts.

41. (original) A method as set forth in claim 27 wherein said dissolved oxygen content is established by bubbling an oxygen-containing gas through said sporulation medium.

42. (original) A method as set forth in claim 41 wherein said oxygen-containing gas consists essentially of air.

43. (original) A method as set forth in claim 41 wherein said gas comprises commercially pure oxygen.

44. (original) A method as set forth in claim 27 further comprising maintaining the temperature from a temperature that substantially avoids freezing to about 45° C.

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45. (original) A method as set forth in claim 44 wherein temperature is maintained from about 15° C to about 40° C.

46. (original) A method as set forth in claim 45 wherein temperature is maintained from about 20° C to about 30° C.

47. (original) A method as set forth in claim 46 wherein temperature is maintained at about 28° C.

48. (original) A method as set forth in claim 27 further comprising incubating the oocysts under said conditions from about 72 hours to about 120 hours.

49. (original) A method as set forth in claim 48 wherein the oocysts incubate from about 72 hours to about 96 hours.

50. (original) A method as set forth in claim 49 wherein the oocysts incubate for about 72 hours.

51. (original) A method as set forth in claim 27 further comprising controlling the pH of the sporulation medium.

52. (original) A method as set forth in claim 51 wherein the pH is controlled by the introduction of an acid or base to the sporulation medium.

53. (original) A method as set forth in claim 52 wherein the pH of the sporulation medium is controlled by alternatively

adding sodium hydroxide and sulfuric acid to the sporulation medium.

54. (original) A method as set forth in claim 53 wherein the pH of the sporulation medium is controlled from about 7.2 to about 7.5.

55. (original) A method as set forth in claim 54 wherein the pH of the sporulation medium is controlled at about from 7.35 to about 7.45.

56. (original) A method as set forth in claim 38 wherein said sporulated oocysts are separated from said sporulation medium by filtration or by centrifugal-based separation.

57. (original) A method as set forth in claim 56 wherein said sporulated oocysts are separated by filtration.

58. (original) A method as set forth in claim 57 wherein said sporulated oocysts are separated from the sporulation medium by tangential flow filtration.

59. (original) A method as set forth in claim 38 wherein said sterilization is achieved by adding a chemical disinfectant to sporulated oocysts separated from said sporulation medium.

60. (original) A method as set forth in claim 59 wherein said sterilization substantially eliminates microorganisms.

61. (original) A method as set forth in claim 60 wherein said microorganisms are selected from the group comprising infectious bursal disease virus and chicken anemia virus.

62. (original) A method as set forth in claim 59 wherein said sterilization is by a chemical disinfectant other than an alkali metal dichromate.

63. (original) A method as set forth in claim 59 wherein said chemical disinfectant comprises a solution of an alkali metal hypochlorite.

64. (original) A method as set forth in claim 63 wherein said chemical disinfectant comprises a solution of sodium hypochlorite.

65. (original) A method as set forth in claim 64 wherein said solution used is at a concentration from about 1% to about 20% by volume of active chlorine.

66. (original) A method as set forth in claim 65 wherein said is at a concentration from about 5% to about 15% by volume of active chlorine.

67. (original) A method as set forth in claim 66 wherein said solution is at a concentration of about 10% by volume of active chlorine.

68. (original) A method as set forth in claim 64 wherein said sporulated oocysts are treated with said sodium hypochlorite from about 5 to about 25 minutes.

69. (original) A method as set forth in claim 68 wherein said sporulated oocysts are treated with said sodium hypochlorite from about 8 to about 20 minutes.

70. (original) A method as set forth in claim 69 wherein said sporulated oocysts are treated with said sodium hypochlorite for about 10 minutes.

71. (original) A method as set forth in 63 further comprising substantially separating said sodium hypochlorite from the sporulated oocysts by filtration.

72. (original) A method as set forth in claim 71 wherein said filtration is by means of tangential flow filtration.

73. (original) A method for inducing sporulation of oocysts comprising:

introducing into an aqueous sporulation medium oocysts of at least one species of protozoa known to cause coccidiosis;

incubating said oocysts in said aqueous sporulation medium, thereby causing sporulation of oocysts; and

introducing an oxidizing agent having a standard reduction potential of at least about 0.5 V into said medium at a rate

sufficient to maintain the oxidation potential of said medium equivalent to the oxidation potential of a medium containing dissolved molecular oxygen in concentration of at least 30% of saturation during sporulation;

said medium containing less than about 0.8% by weight alkali metal dichromate during incubation of said oocysts.

74. (original) A method for sporulating oocysts comprising:

introducing into an aqueous sporulation medium oocysts of at least one species of protozoa known to cause coccidiosis;

incubating said oocysts in said aqueous sporulation medium, thereby causing sporulation of oocysts; and

separating said oocysts by tangential flow filtration from said sporulation medium.

75. (original) A method for sterilizing oocysts comprising:

contacting oocysts of at least one species of protozoa known to cause coccidiosis with a sterilization medium; and

removing said sterilization medium from said oocysts by tangential flow filtration.

76. (original) A method for monitoring sporulation of oocysts comprising:

incubating viable oocysts in an aqueous sporulation medium; and

during incubation, monitoring said medium to detect a change in at least one of the following parameters:

- (i) dissolved oxygen content;
- (ii) pH;
- (iii) rate of introduction of oxidizing agent into said medium;
- (iv) flow rate of acid or base into said medium.

77. (original) A method as set forth in claim 76 wherein dissolved oxygen content of said medium is controlled by addition of molecular oxygen thereto, and monitoring sporulation comprises detecting a change in oxygen consumption as indicated by detection of a change in oxygen flow to the medium and/or a permanent or transient change in dissolved oxygen content.

78. (original) A method as set forth in claim 76 wherein pH of said medium is controlled by addition of acid or base thereto, and monitoring sporulation comprises detecting an increase in acid consumption as indicated by an increase in acid flow to the medium and/or a permanent or transient increase in pH.

79. (original) A method as set forth in claim 76 wherein the end point of sporulation is determined from substantial cessation of oxygen consumption or generation of alkalinity in the sporulation medium.

80. (original) A method as set forth in claim 79 wherein the end point is indicated by the substantial cessation of change in at least one of said parameters.

81. (original) A method as set forth in claim 79 wherein said sporulated oocysts are maintained in said medium under sporulation conditions for at least another 48 hours after the indicated end point of sporulation.

82. (original) A method as set forth in claim 76 wherein said change in dissolved oxygen content is a decrease.

83. (original) A method as set forth in claim 76 wherein said change in pH is an increase.

84. (original) A method as set forth in claim 83 wherein said increase in pH is at least 0.5 pH units.

85. (original) A method as set forth in claim 84 wherein said increase in pH is at least 0.25 pH units.

86. (original) A composition for the storage of sporulated oocysts comprising an aqueous diluent and a bactericide, said composition characterized as substantially free of alkali metal dichromate wherein said composition is characterized as having:  
a diluent comprising 0.5X phosphate buffered saline;  
a pH from about 5.0 to about 8.0; and



wherein said bactericide is selected from the group consisting of an alkali metal perchlorate, an alkali metal hypochlorite, hydrochlorous acid, sodium hydroxide and antibiotics.

87. (original) A composition as set forth in claim 86 having a pH from about 7.0 to about 7.5.

88. (original) A composition as set forth in claim 86 wherein said bactericide comprises gentamicin.

89. (original) A composition as set forth in claim 86 further comprising an oxidizing agent.

90. (original) A composition as set forth in claim 86 characterized in that an oocyst population in contact with said composition remains at least about 60% viable for 13 weeks at about 25° C.

91. (original) A composition as set forth in claim 86 characterized in that an oocyst population in contact with said composition remains at least about 60% viable for 26 weeks at about 5°C.

92. (original) A composition as set forth in claim 86 characterized in that an oocyst population in contact with said composition decrease in viability no more than about 20% over a period of at least about 13 weeks at about 25°C.

93. (original) A composition as set forth in claim 86 characterized in that an oocyst population in contact with said composition decrease in viability no more than about 20% over a period of at least about 26 weeks at about 5°C.

94. (original) A composition as set forth in claim 86 further comprising a dye.

95. (original) A composition for the storage of sporulated oocysts comprising:

0.5X PBS; and

about 30 µg/ml gentamicin,

said composition characterized as substantially free of alkali metal dichromate, and wherein said composition is characterized in that oocysts in contact with said

composition decrease in viability no more than about 20% over a period of at least about 26 weeks at about 5°C.

96. (original) A method for storing sporulated oocysts comprising contacting said sporulated oocysts with the composition of claim 86.

97. (original) A method as set forth in claim 96 further comprising storing said sporulated oocysts in contact with the composition of claim 86 at either about 25°C or about 5°C.

98. (original) A method as set forth in claim 96 wherein said population of sporulated oocysts is maintained at least 60% viable for 13 weeks at about 25°C.

99. (original) A method as set forth in claim 96 wherein said population of sporulated oocysts is maintained at least 60% viability for 26 weeks at about 5°C.

100. (original) A method as set forth in claim 96 wherein said method prevents a decrease in oocyst viability of greater than 20% over a period of at least 13 weeks at about 25°C.

101. (original) A method as set forth in claim 96 wherein said method prevents a decrease in viability of greater than 20% in a population of sporulated oocysts over a period of at least 26 weeks at about 5°C.